Bio-Rad SARS-CoV-2 ddPCR Kit

Instructions For Use

SARS-CoV-2 ddPCR Kit
Part Number 12013743

Qualitative assay for use on the QX200
Droplet Digital PCR Systems

For Research Use Only

10000130776 Ver B
May 2020

UNITED STATES, Bio-Rad Laboratories, Inc., 5731 W. Las Positas Blvd.,
Pleasanton, CA 94588, 510-724-7000

EC REP FRANCE, Bio-Rad, 3 boulevard Raymond Poincaré, 92430
Marnes-la-Coquette, France, Tel: +33 (0)1 47 95 60 00

TRANSLATIONS

Product documents may be provided in additional languages on electronic media.

SYMBOLS LEXICON

CE European Conformity	Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	Use by	IVD For In Vitro Diagnostic Use
Temperature Limit	REF Catalog Number	Consult Instructions for Use
Number of Tests	USE For use with	SN Serial Number
Rx Only Precription Use Only	LATEX Contains Latex	UDI-DI Unique Device Identification – Device Identifier

BIO-RAD TECHNICAL SUPPORT

For help and technical advice, please contact the Bio-Rad Technical Support department. In the United States, the Technical Support department is open Monday–Friday, 5:00 AM–5:00 PM, Pacific time.

Phone: 1-800-2BIORAD (1-800-224-6723)

Online technical support and worldwide contact information are available at www.consult.bio-rad.com

LEGAL NOTICES

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage or retrieval system, without permission in writing from Bio-Rad Laboratories.

Bio-Rad reserves the right to modify its products and services at any time. This instruction manual is subject to change without notice. Although prepared to ensure accuracy, Bio-Rad assumes no liability for errors, or for any damages resulting from the application or use of this information.

BIO-RAD, DDPCR, DROPLET DIGITAL, DROPLET DIGITAL PCR, and QUANTASOFT are trademarks of Bio-Rad Laboratories, Inc. in certain jurisdictions.

All trademarks used herein are the property of their respective owner.

This product and/or its use is covered by claims of U.S. patents, and/or pending U.S. and non-U.S. patent applications owned by or under license to Bio-Rad Laboratories, Inc. Purchase of the product includes a limited, non-transferable right under such intellectual property for use of the product for internal research and diagnostic purposes only. For the purposes of COVID-19 only, Bio-Rad grants the rights for use of the product for commercial applications of any kind, including but not limited to manufacturing, quality

control, or commercial services, such as contract services or fee for services. Information concerning a license for such uses can be obtained from Bio-Rad Laboratories. For any purpose beyond COVID-19 testing, it is the responsibility of the purchaser/end user to acquire any additional intellectual property rights that may be required.

Purchase of Digital PCR products from Bio-Rad Laboratories includes a limited, non-transferable right under our intellectual property for you to use the products for your internal research purposes only. Unless we expressly state otherwise in additional Terms and Conditions, no rights are granted for you to distribute or resell the products or to use the products for commercial applications, such as contract services or fee for services. No rights are granted for diagnostic uses. Information concerning a license for such uses can be obtained from Bio-Rad Laboratories. It is the responsibility of the purchaser/end user to acquire any additional intellectual property rights that may be required. The QX ONE/QX200/QX100/QX200 AutoDG Droplet Digital PCR Systems and/or their use is covered by claims of U.S. patents, and/or pending U.S. and non-U.S. patent applications owned by or under license to Bio-Rad Laboratories, Inc. See bio-rad.com/en-us/trademarks for details.

SAFETY AND REGULATORY COMPLIANCE

QX200 AutoDG ddPCR System has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

1. IEC 61010-1:2010 (3rd ed.), EN61010-1:2010 (3rd ed). Electrical Equipment for Measurement, Control, and Laboratory Use — Part 1: General Requirements
2. EN 61326-1:2006 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General Requirements
3. UL 61010-1:2004, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls
4. CAN/CSA 22.2 No 61010-1-04, Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I: General Requirements

This equipment generates, uses, and can radiate radiofrequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense.

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.

The CSA mark indicates that a product has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards.

The Waste Electrical and Electronic Equipment Directive symbol indicates that when the end- user wishes to discard this product, it must be sent to separate collection facilities for recovery and recycling.

Bio-Rad SARS-CoV-2 ddPCR Kit Warnings and Precautions

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.

Position the equipment near a grounded outlet with the plug of the power supply accessible. The plug of the power supply is the disconnect device.

Two people are required to lift the QX200 or QX200 Automated Droplet Generator. Grip from the underside, one person on each side.

No serviceable parts inside.

PPE (Personal Protective Equipment) Training

Proper use of gloves is recommended with use of oils and sample plates. OSHA requirements for PPE are set forth in the Code of Federal Regulations (CFR) at 29 CFR 1910.132 (General requirements); 29 CFR 1910.138 (Hand protection); 29 CFR 1926.95 (Criteria for standard personal protective equipment). Any gloves with impaired protective ability should be discarded and replaced. Consider the toxicity of the chemicals and factors such as duration of exposure, storage, and temperature when deciding to reuse chemically exposed gloves. Features to aid glove selection for handling of machines, assays, oils, and cleaning solvents:

Butyl gloves are made of a synthetic rubber and protect against peroxide, hydrofluoric acid, strong bases, alcohols, aldehydes, and ketones.

Natural (latex) rubber gloves are comfortable to wear and feature outstanding tensile strength, elasticity, and temperature resistance.

Neoprene gloves are made of synthetic rubber and offer good pliability, finger dexterity, high density, and tear resistance; they protect against alcohols, organic acids, and alkalis.

Nitrile gloves are made of copolymer and provide protection from chlorinated solvents such as trichloroethylene and tetrachloroethene; they offer protection when working with oils, greases, acids, and caustic substances.

Table of Contents

Bio-Rad Technical Support ii
Legal Notices ii
Safety and Regulatory Compliance iii
Bio-Rad SARS-CoV-2 ddPCR Kit Warnings and Precautions iii
PPE (Personal Protective Equipment) Training iv
Principle of Procedure 3
Bio-Rad SARS-CoV-2 ddPCR Kit Workflow 3
Reagents & instruments 5
Materials Provided 6
Materials Required but Not Provided 7
General Precautions and Warnings 7
Specimen Collection, Transport and Storage 8
Reagent Preparation and Storage 8
Control Materials 9
Nucleic Acid Extraction 10
General Handling 10
Bio-Rad SARS-CoV-2 ddPCR Test Protocol 11
Overview 11
Description of Test Steps 11
Preparation of One-Step RT-ddPCR Reactions 11
RT- ddPCR (C1000 Touch or S1000 with 96-Deep Well Reaction Module Thermal Cyclers) 16
Droplet Reading, Data Acquisition and Analysis 17
References 26

Trade Name: Bio-Rad SARS-CoV-2 ddPCR Test

PRINCIPLE OF PROCEDURE

The Bio-Rad SARS-CoV-2 ddPCR Kit is a reverse transcription (RT) droplet digital polymerase chain reaction (ddPCR) test designed to detect RNA from the SARS-CoV-2 in nasopharyngeal anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens from individuals who are suspected of COVID-19 by their healthcare provider.

The oligonucleotide primers and probes for detection of SARS-CoV-2 are the same as those reported by CDC and were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel. The Bio-Rad SARS-CoV-2 ddPCR Kit includes these three sets of primers/probes into a single assay multiplex to enable a one-well reaction.

RNA isolated and purified from nasopharyngeal, anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens is added to the mastermix comprised of reverse transcriptase whereby RNA is converted into cDNA and then amplified, using the Bio-Rad One-Step RT-ddPCR supermix in the Kit.

The sample and mastermix RT-ddPCR mixtures are fractionated into up to 20,000 nanoliter-sized droplets in the form of a water-in-oil emulsion in the Automated Droplet Generator. The emulsions are then thermocycled to achieve reverse transcription to generate cDNA followed by target amplification plus probe hydrolysis in each droplet. Subsequent to PCR, the fluorescence intensity of each droplet is measured in two channels (FAM and HEX) in the Droplet Reader. The fluorescence data is then analyzed by the QuantaSoft v1.7 Software and QuantaSoft Analysis Pro v1.0 Software to determine the presence of SARS-CoV-2 N1 and N2 in the specimen.

Twenty-two microliters (22µl) of each RT-ddPCR ready sample are loaded into the wells of a 96-well PCR plate. The plate and required consumables are loaded into the QX200 Automated Droplet Generator. The consumables required are: Automated Droplet Generation Oil, Automated Droplet Generator plates, cartridges, and pipet tips. The QX200 Automated Droplet Generator uses microfluidics to combine oil and an aqueous sample to generate the nanoliter-sized droplets required for ddPCR analysis.

The 96-well RT-ddPCR ready plate containing droplets from the QX200 Automated Droplet Generator is sealed with foil using a plate sealer, and subjected to thermocycling using either the Bio-Rad C1000 Touch or S1000 Thermocyclers.

After thermocycling is complete, the 96-well RT-ddPCR ready plate is loaded into the QX200 Droplet Reader. The Droplet Reader singulates the droplets and flows them past a two-color fluorescence detector. The detector reads the droplets to determine which contain target (positive) and which do not (negative) for each of the targets identified with the SARS-CoV-2 ddPCR Test: N1, N2 and RP. The ddPCR system uses QuantaSoft v1.7 and QuantaSoft Analysis Pro v1.0 for analysis software.

BIO-RAD SARS-COV-2 DDPCR KIT WORKFLOW

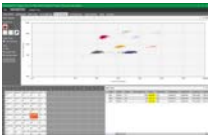
The SARS-CoV-2 ddPCR Kit is intended for use on Bio-Rad QX200 AutoDG Droplet Digital PCR (ddPCR) System (Table 1) and the workflow consists of five steps (Figure 1).

Table 1: Instruments Required

Status	Part Number	Product Name
RUO System	1864100 (Top level part number)	QX200 AutoDG Droplet Digital PCR System consisting of:
	1864003	QX200 Droplet Reader
	1864101	Automated Droplet Generator
RUO Lab Equipment	1851197	C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module
	1852197	S1000 Thermal Cycler with 96-Deep Well Reaction Module
	1814000	PX1 PCR Plate Sealer

Figure 1: SARS-CoV-2 ddPCR Test Workflow for 96 samples

	Workflow	Instrument	Estimated Hands-on Time	Estimated Instrument Time
Step 1	Isolation of viral RNA from anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens swabs	ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit or QIAgen QIAamp Viral RNA Mini Kit	<60 min.	N/A
Step 2	• RT-ddPCR plate setup • Droplet generation		<10 min.	~45 min
Step 3	One-step reverse transcription and PCR amplification	or	<10 min.	~210 min
Step 4	Droplet reading		<5 min.	~120 min

	Workflow	Instrument	Estimated Hands-on Time	Estimated InstrumentTime
Step 5	Analysis		< 5 min	< 1 min

REAGENTS & INSTRUMENTS

Materials Provided

Table 2: Materials Required for the SARS-CoV-2 ddPCR Test

Part Number	Name	Reactions	Storage Conditions
12013743	SARS-CoV-2 ddPCR Kit	600	–25°C to –15°C
10000130776	SARS-CoV-2 ddPCR Test Instructions For Use		

NOTE: Safety Data Sheets (SDS) are available at www.bio-rad.com.

Materials Required but Not Provided

Reagents for RNA Purification

The ThermoFisher MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat No. A48310, 1000 reactions) and the QIAamp Viral Mini Kit (Cat No. 52906, 250 reactions) were validated for use with the SARS-CoV-2 ddPCR Kit per the manufacturer's instructions.

Generic Reagents and Consumables for Droplet Digital PCR

Table 3: Materials Required but not provided for running on the QX200 ddPCR System

Part Number	Name	Quantity (each)	Volume	Storage Conditions
12003015	ddPCR Pierceable Foil Seals	50	N/A	15°C to 30°C
12003185	ddPCR 96 Well Plates	15	N/A	15°C to 30°C
12003016	DG32 Cartridges w/ Gaskets	15	N/A	15°C to 30°C
12003010	Pipet Tips for AutoDG (Racks)	10	N/A	15°C to 30°C
12003017	AutoDG Oil for Probes	1	50 mL	15°C to 30°C
1863052	ddPCR Buffer Control for Probes	1	2 x 4.5 mL	15°C to 30°C

Table 4: Materials Required but not provided for running on the QX200 ddPCR System

Part Number	Name	Quantity (each)	Volume	Storage Conditions
1814040	ddPCR Pierceable Foil Seals	50	N/A	15°C to 30°C
12001925	ddPCR 96 Well Plates	15	N/A	15°C to 30°C
1864108 1864109	DG32 AutoDG Cartridges w/ Gaskets	15	N/A	15°C to 30°C
1864120 1864121	Pipet Tips for AutoDG (Racks)	10	N/A	15°C to 30°C
1864125	Waste Bins for AutoDG		N/A	15°C to 30°C
1864110	AutoDG Oil for Probes	1	50 mL	15°C to 30°C
1864008 1864007	DG8 Cartridges	1	N/A	15°C to 30°C
1863009 1864007	DG8 Gaskets	1	N/A	15°C to 30°C
1863005	DG Oil for Probes	1	50 mL	15°C to 30°C
1863004	ddPCR Droplet Reader Oil	1	1L	15°C to 30°C
1863052	ddPCR Buffer Control for Probes	1	2 x 4.5 mL	15°C to 30°C

Instrumentation, Software and General Laboratory Equipment

Table 5: Instruments required but not provided in the Bio-Rad SARS-CoV-2 ddPCR Kit

Status	Part Number	Product Name
RUO System	1864100	QX200 AutoDG Droplet Digital PCR System consisting of:
	1864003	QX200 Droplet Reader
	1864101	Automated Droplet Generator
RUO Lab Equipment	1851197	C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module
	1852197	S1000 Thermal Cycler with 96-Deep Well Reaction Module
	1814000	PX1 PCR Plate Sealer

Table 6: General Laboratory Equipment required but not provided in the Bio-Rad SARS-CoV-2 ddPCR Kit

Description
Adjustable pipettors (Rainin or Eppendorf) • 1 – 10 µL • 10– 100 µL • 20 – 200 µL • 100 – 1000 µL
Adjustable 8-channel pipettors (Rainin or Eppendorf) • 10 – 50 µL, 10 – 100 µL, or 20 – 200 µL
Microcentrifuge
Microwell plate Centrifuge
Vortex mixer

GENERAL PRECAUTIONS AND WARNINGS

- For professional use only.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- All biological specimens should be treated as if they are capable of transmitting infectious agents using safe laboratory procedures, such as those outlined in HHS Publication (CDC) 21-1112, Biosafety in Microbiological and Biomedical Laboratories and in CLSI Document M29-A4, Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.[1, 2]
- Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite (10% bleach) in deionized or distilled water, followed by 70% alcohol.
- To minimize nucleic acid contamination routinely decontaminate bench space, pipettors and equipment, and separate the specimen and RNA/DNA handling area from the assay preparation area.
- Optimize workflow and space to minimize risk of carry-over contamination from completed PCR reactions.
- Ensure that Automated Droplet Generator and Droplet Reader have a dedicated space in separate areas to avoid amplicon contamination.
- Perform assay setup and template addition in different locations, with dedicated pipettors.
- Use proper laboratory safety procedures for working with chemicals and handling specimens.
- Change gloves frequently when transporting and working with different reagents.

- Failure to follow the procedures and conditions described in this document can cause incorrect results and adverse effects.
- Do not substitute Bio-Rad SARS-CoV-2 ddPCR Test reagents with other reagents.
- Setup and template addition must be performed under RNAse/DNAse-free conditions.
- Ensure that regular maintenance and calibration is performed on all equipment according to manufacturer's recommendations.
- Use nuclease-free tips and reagents, and routinely clean pipettors.
- Ensure that only the recommended thermal cycling protocol is used.
- Do not use DEPC treated water for PCR amplification.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Adequate, appropriate specimen collection, storage, and transport are important in order to obtain sensitive and accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results. CLSI MM13-A may be referenced as an appropriate resource.

1. Sample acceptance criteria
   - Samples should be collected into sterile, labeled tubes, and shipped at 2°C to 8°C on frozen gel packs.
2. Specimen rejection criteria
   - Samples that have not been pre-approved for testing and those that are labeled improperly will not be tested until the required information is obtained.
3. Collecting the Specimen
   - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
   - Follow specimen collection devices manufacturer instructions for proper methods.
   - Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron® and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media or universal transport media.
4. Transporting Specimens
   - Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens to the testing laboratory.
   - Store specimens at 2-8°C and ship overnight to the testing laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to the testing laboratory on dry ice.

5. Storing Specimens
   - Specimens can be stored at 2-8°C for up to 72 hours after collection.
   - If a delay in extraction is expected, store specimens at -70°C or lower.
   - Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or -70°C or lower if stored longer than 4 hours.

REAGENT PREPARATION AND STORAGE

SARS-CoV-2 ddPCR Kit

- Store at 4°C, long term storage at -20°C is recommended.
- Thaw all components on ice for 30 min.
- Mix thoroughly by vortexing each tube at maximum speed for 30 sec to ensure homogeneity because a concentration gradient may form during -20°C storage.
- Centrifuge briefly to collect contents at the bottom of each tube.
- Prepare the reaction mix aliquot for the number of reactions required (Table 7).
- Assemble all required components except the sample, dispense equal aliquots into each reaction well, and add the sample to each reaction well as the final step.
- The reactions should be set up on ice before droplet generation and the RT enzyme must be stored at -20°C at all times or on ice when being used.

CONTROL MATERIALS

Controls provided with the Bio-Rad SARS-CoV-2 ddPCR Kit:

1. A “no template” (negative) control is needed to detect reagent and/or environmental contamination, in which RNase/DNase-free water is used in lieu of a clinical specimen sample, per each sample extraction batch, with a minimum of one well per every 96-well plate.
2. A positive template control is needed to detect substantial reverse transcriptase process and/or reagent failure including primer and probe integrity. The test will utilize Exact Diagnostic’s SARS-CoV-2 Standard which is manufactured with synthetic RNA transcripts containing five gene targets: E, N, ORF1ab, RdRP and S Genes of SARS-CoV-2, each quantitated at 200,000 cp/ml along with human genomic DNA background. The material is spiked into a sample-like matrix that requires nucleic acid extraction, identical to a clinical patient specimen (100 uL aliquot per extraction). The positive control will be used at a concentration close to the limit of detection (<5x LoD) of the test and is run like a clinical specimen. One positive template control must be included per each sample extraction batch, with a minimum of one well per every 96-well plate.
3. An extraction control is needed to detect extraction step failure. The test will utilize Exact Diagnostic’s SARS-CoV-2 Negative which is manufactured with human genomic RNA and DNA spiked into a sample-like matrix that requires nucleic acid extraction, identical to a clinical patient specimen (100 uL aliquot per extraction). One extraction control must be included per each sample extraction batch, with a minimum of one well per every 96-well plate.

NUCLEIC ACID EXTRACTION

All necessary safety precautions should be taken according to the Laboratory guidelines. Precautions must also be taken to prevent cross contamination of samples. Separate work areas should be used for:

- Nucleic acid extraction
- Reagent preparation (e.g., preparation of RT-ddPCR master mix; NO amplified reactions, target solutions, or clinical specimens should be brought into this area. After working in this area, laboratory coat and gloves should be changed before moving into the nucleic acid addition area)
- Nucleic acid addition
- Instrumentation (e.g., thermocyclers)

General Handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed. During the procedure, work quickly and keep everything on cold blocks when possible to avoid degradation of RNA by endogenous or residual RNases. Clean working surfaces, pipettes, etc. with 20% bleach or other solution that can destroy nucleic acids and RNases. To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach. Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents.

Performance of the Bio-Rad SARS-CoV-2 ddPCR Test is dependent upon the amount and quality of template RNA purified from human specimens. The following commercial extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the test:

ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Cat No. A48310, 1000 reactions)

- Add patient sample to an input volume of 100µl and elute with 75µL of RNase/DNase-free water following the manufacturer's instructions.

QIAamp Viral RNA Mini Kit (Cat No. 52906, 250 reactions)

- Add patient sample to an input volume of 140µl and elute with 75µL of AVE buffer or RNase/DNase-free water following the manufacturer's instructions.

Extracted nucleic acid should be stored at 4°Cif it is to be used within 4 hours, or at –70°C if stored longer than 4 hours.

Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for sample extraction. No template control (NTC) and extraction control must be included in each extraction batch.

BIO-RAD SARS-CoV-2 ddPCR TEST PROTOCOL

Overview

The SARS-CoV-2 ddPCR Test is intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in respiratory specimens (nasopharyngeal swabs, anterior nasal and mid-turbinate swabs as well as nasopharyngeal wash/aspirate and nasal wash/aspirate). The assay targets regions of the virus nucleocapsid gene (N1 and N2) and is designed for the specific detection of SARS-CoV-2. Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

The test is composed of three principal steps: (1) extraction of RNA from patient specimens, RT-PCR reaction preparation and droplet generation, (2) one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and ddPCR detection with the SARS-CoV-2 specific Taqman probes and (3) ddPCR droplet reading on the QX200 Droplet Reader.

Description of Test Steps

Nucleic acids are isolated and purified from nasopharyngeal, anterior nasal and mid-turbinate swabs, nasopharyngeal wash/aspirate and nasal wash/aspirate specimens using the ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, or the QIAgen QIAamp Viral RNA Mini Kit following the manufacturer's instructions for use. The purified nucleic acids are reverse transcribed and amplified using Bio-Rad's SARS-CoV-2 ddPCR Kit, which includes three supermix components: Supermix, Reverse Transcriptase and DTT. The reaction mastermix preparation is described in Table 7. The SARS-CoV-2 ddPCR Assay contains the primers and probes for SARS-CoV-2 targets N1 and N2 as well as the Human RNase P primers and probes in a single tube so that it can be used as a single well test.

Table 7: Preparation of the Reaction Mastermix

Component	Volume per Reaction, µl	Final Concentration
Supermix	5.5	1x
Reverse transcriptase	2.2	20U/µl
300 mM DTT	1.1	15mM
SARS-CoV-2 ddPCR Assay (20x)	1.1	1x
RNA sample	5.5	100fg-100ng per reaction
RNase/DNase free water	6.6	--
Volume per reaction*	22	

* Volumes include 10% excess in setup.

Preparation of One-Step RT-ddPCR Reactions

NOTE: Ensure that the bench space has been properly cleaned with 10% bleach and 70% alcohol.

NOTE: Repeated freezing and thawing of the supermix is not recommended. DTT should be aliquoted to multiple tubes and stored at -20°C to minimize freezing and thawing.

1. Ensure extracted RNA sample(s) are thawed.
   NOTE: Do not vortex RNA samples. RNA samples may be mixed by flicking the tubes, followed by brief centrifugation to collect the contents to the bottom of the tubes.
2. Place the Reverse Transcriptase on ice or cold block.
3. Bring the SARS-CoV-2 ddPCR Assay to room temperature.
4. Thaw the following components at room temperature for up to 15 minutes.
   a. One-Step Supermix
   b. 300 mM DTT
   c. Nuclease-free water
5. Vortex each component in step 4, and briefly centrifuge to collect contents to the bottom of the tube.
6. Place the components on ice or cold block.
7. RT Master Mix Preparation:
   a. Prepare Master Mix according to the number of patient samples and controls to be tested (Table 8).
   b. Vortex the Master Mix for 30 seconds, and centrifuge briefly to collect the contents to the bottom of the tube.

NOTE: The One-Step RT-ddPCR Supermix is extremely viscose. It is critical to vortex at high speed for at least 30 seconds after thawing and before beginning the assay mix preparation. It is recommended to vortex the assay mix as it is being prepared after each addition of reagent. Quick-centrifuge to spin down and collect the solution before adding the next component. Finally, vortex the prepared reaction mix for 30 seconds at high speed before plating.

Table 8: RT-ddPCR Master Mix Component Volumes by Number of Patient Samples

	# of Samples* (samples + overage) Volume µL				
Component	1x	(16+2)x	(32+3)x	(48+4)x	(96+6)x
Supermix	5.5	99	192.5	286	561
Reverse transcriptase	2.2	39.6	77	114.4	224.4
300 mM DTT	1.1	19.8	38.5	57.2	112.2
SARS-CoV-2 ddPCR Assay	1.1	19.8	38.5	57.2	112.2
Nuclease-free water	6.6	118.8	231	343.2	673.2
Total	16.5	297	577.5	858	1683

8. Dispense 16.5 µL of the Master Mix into the appropriate wells of the RT-ddPCR plate. Add 5.5µL of the extracted RNA sample per the suggested plate layout (Figure 2).
9. Add 5.5 µL of the ExactDx Standard and ExactDx Negative (Figure 2)
   NOTE: The RT-ddPCR plate may be placed on ice while loading.
   NOTE: RT-ddPCR plates may not contain incomplete columns. If any columns are partially used, follow step 10.

10. If all eight wells in a column are not used for controls or samples, ddPCR Buffer Control (ddPCR™ Buffer Control for Probes diluted 1:1 with water) must be added to the unused wells. The Automated Droplet Generator requires all wells in a column to contain a PCR reaction or buffer. Droplets will not form if any wells in a column are empty.

Figure 2: RT-ddPCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	PtSample6	PtSample14	PtSample22	PtSample30	PtSample38	PtSample46	PtSample54	PtSample62	PtSample70	PtSample78	PtSample86
B	ExactDxNegExtCtrl	PtSample7	PtSample15	PtSample23	PtSample31	PtSample39	PtSample47	PtSample55	PtSample63	PtSample71	PtSample79	PtSample87
C	ExactDxPosExtCtrl	PtSample8	PtSample16	PtSample24	PtSample32	PtSample40	PtSample48	PtSample56	PtSample64	PtSample72	PtSample80	PtSample88
D	PtSample1	PtSample9	PtSample17	PtSample25	PtSample33	PtSample41	PtSample49	PtSample57	PtSample65	PtSample73	PtSample81	PtSample89
E	PtSample2	PtSample10	PtSample18	PtSample26	PtSample34	PtSample42	PtSample50	PtSample58	PtSample66	PtSample74	PtSample82	PtSample90
F	PtSample3	PtSample11	PtSample19	PtSample27	PtSample35	PtSample43	PtSample51	PtSample59	PtSample67	PtSample75	PtSample83	PtSample91
G	PtSample4	PtSample12	PtSample20	PtSample28	PtSample36	PtSample44	PtSample52	PtSample60	PtSample68	PtSample76	PtSample84	PtSample92
H	PtSample5	PtSample13	PtSample21	PtSample29	PtSample37	PtSample45	PtSample53	PtSample61	PtSample69	PtSample77	PtSample85	PtSample93

11. After adding template to the master mix plate, cover the plate with a foil seal using the PX1 Plate Sealer.
    a. Set the PX1 plate sealer to 180°C for 5 seconds (not the default conditions)
    b. Keep the aluminum block at room temperature while the sealer is not in use.
    c. Place the ddPCR plate on the room temperature block.
    d. Cover the plate with one sheet of pierceable foil seal. The red stripe should be visible.

       NOTE: Do not use the metal frame.

    e. Touch the Seal button to seal the plate (this will close the door, and initiate heat sealing).
    f. Remove the plate and aluminum block from PX-1 plate sealer.
    g. Check that all of the wells on the plate are sealed by confirming the depressions of the wells are visible on the foil.

    NOTE: For more detailed instructions refer to PX1 Plate Sealer Instruction Manual.

    NOTE: Remove the aluminum block from PX1 plate sealer while not in use, in order to avoid over-heating the block.

12. Vortex the plate for 30 seconds at high speed.
13. Centrifuge the ddPCR plate for 30 seconds at 1000 rcf to remove any air bubbles and allow the RT-ddPCR reaction mix to pool at the bottom of the wells. If bubbles remain, spin the plate again.
14. Proceed with droplet generation.

Droplet Generation

NOTE: The instructions included in this section are applicable for QX200 AutoDG Droplet Digital PCR Systems.

NOTE: Ensure that the bench space and instrument surface area have been properly cleaned with 10% bleach and 70% alcohol.

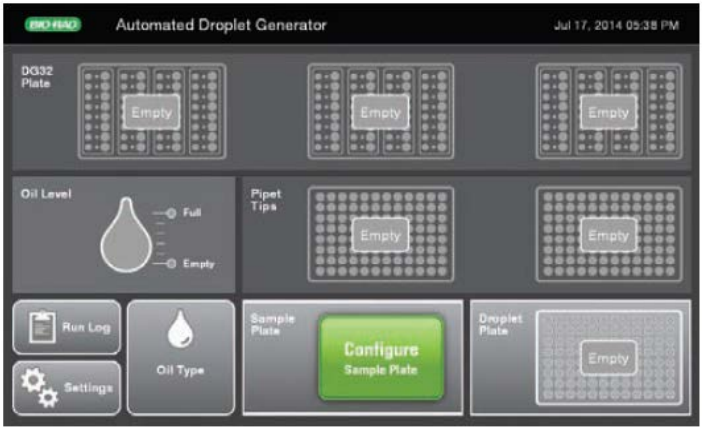
NOTE: Ensure that the Trash container is empty and is sitting in the appropriate location on the AutoDG.

NOTE: Ensure that the cooling block accessory is stored upside down at -20°C for at least 2 hours before starting droplet generation.

NOTE: Periodically inspect the cooling block accessory to ensure that the foil seal is intact.

1. Gather all of the consumable materials needed to setup the QX200 Automated Droplet Generator.
    a. DG32 Cartridges
    b. Pipet Tips for AutoDG (racks)
    c. AutoDG Oil for probes
    d. Sample Plate (RT-ddPCR plate containing the reactions)
    e. Droplet Plate (new ddPCR plate into which the generated droplets will be dispensed)
2. Open the AutoDG door and load the Sample plate onto the Automated Droplet Generator in the Sample Plate position. The indicator light should turn green (Figure 3).

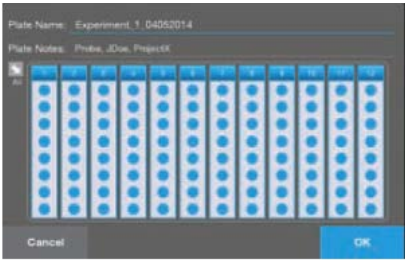
Figure 3: Automated Droplet Generator System User Interface



3. Touch the Configure Sample Plate button on the AutoDG interface, select the columns in which the samples are located, and touch “OK.” Plate name and plate notes may be entered, but are not required (Figure 4).

    NOTE: Wells can only be selected in columns of 8 on the AutoDG Configure Screen (Figure 4).

Figure 4: Sample Plate Configuration on the AutoDG Interface

A. 12 columns selected (96 wells, 93 samples, 3 controls)	B. 6 columns selected (48 wells, 45 samples, 3 controls)
	

4. Based on the number of columns selected on the sample plate, yellow indicator lights will light up indicating consumables that need to be loaded on the instrument.
5. Remove the cooling block accessory from the freezer (-20°C) and place in the Droplet Plate location on the Automated Droplet Generator (Figure 3). The indicator light should turn green.
6. Load appropriate consumables until associated indicator lights are green.
    a. Load an empty ddPCR 96 well plate onto the cooling block accessory in the Droplet Plate location.
    b. Load the AutoDG Oil for Probes on the left side of the instrument by removing the cap, and twisting the bottle into the tower. Select Probes in the Oil Type area of the AutoDG User Interface, and select OK. The Oil Level icon on the screen will turn blue and display the current oil level in the bottle.

        NOTE: This step is required only if the oil level is not sufficient for the run.
    c. Load AutoDG pipet tips along the center row of the instrument after removing the plastic wrap and box lid.

        NOTE: Only full tip boxes should be loaded on the system.
    d. Load the DG32 cartridges (with green gaskets to the right) along the back row of the instrument into the plate holders. The holders are keyed for proper orientation, and when placed correctly, the light will turn green.
7. Once all of the indicators on the AutoDG are green, touch the blue Start Droplet Generation button.

    WARNING: Stand clear of the instrument, the door closes automatically prior to run initialization.
8. The AutoDG user interface will ask the user to confirm starting the droplet generation run by touching Start Run.

    NOTE: The screen will indicate the time remaining until droplet generation is complete. If droplet formation stops for any reason, a “Run Terminated” message will appear on the AutoDG screen. If the run is terminated, determine the cause of the failure and follow the

instructions in the troubleshooting section of the QX200 AutoDG Droplet Digital PCR System Instruction Manual.

9. Upon successful completion of droplet generation, remove the Droplet Plate, seal it with a foil seal, and start the PCR run.

   NOTE: PCR amplification should begin within 30 minutes of the completion of the AutoDG Droplet Generation run.

10. Cover the plate with a foil seal using the PX1 Plate Sealer.
    a. Set the PX1 plate sealer to 180°C for 5 seconds (not the default conditions).
    b. Keep the aluminum block at room temperature while the sealer is not in use.
    c. Place the ddPCR plate on the room temperature block.
    d. Cover the plate with one sheet of pierceable foil seal. The red stripe should be visible.

       NOTE: Do not use the metal frame.

    e. Touch the Seal button to seal the plate (this will close the door, and initiate heat sealing).
    f. Remove the plate and aluminum block from PX-1 plate sealer.
    g. Check that all of the wells on the plate are sealed by confirming the depressions of the wells are visible on the foil.

    NOTE: For more detailed instructions refer to PX1 Plate Sealer Instruction Manual.

    NOTE: Remove the aluminum block from PX1 plate sealer while not in use, in order to avoid over-heating the block.

11. The Droplet Plate is now ready for the one step RT-PCR reaction.
12. Proceed immediately to RT-ddPCR setup, or store the Droplet Plate at 4°C up to 24 hours.

RT- ddPCR (C1000 Touch or S1000 with 96-Deep Well Reaction Module Thermal Cyclers)

1. Place the sealed Droplet Plate into the thermal cycler for PCR amplification and run the SARS-CoV-2 ddPCR Test Thermal Cycling Protocol (Table 9).

Table 9: SARS-CoV-2 RT-ddPCR Thermal Cycling Protocol

Cycling Step	Temperature (°C)	Time	Number of Cycles
Reverse Transcription	50	60 minutes	1
Enzyme activation	95	10 minutes	1
Denaturation	94	30 seconds	40
Annealing/Extension	55	60 seconds	
Enzyme Deactivation	98	10 minutes	1
Droplet Stabilization	4	30 minutes	1
Hold (optional)	4	24 hours	1

WARNING: It is critical to set the ramp rate to 2°C/second, as default ramp rates differ for different cyclers.

NOTE: When using a Bio-Rad C1000 Touch or S1000 with 96-Deep Well Reaction Module Thermal Cyclers, set the sample volume to 40 µL and lid temperature to 105°C. Refer to the C1000 Touch or S1000 with 96-Deep Well Reaction Module Thermal Cycler Instruction Manual for additional information.

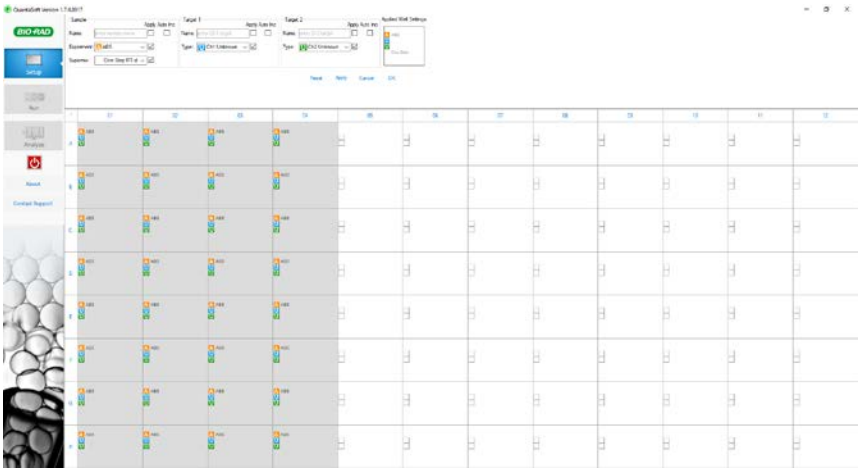
Droplet Reading, Data Acquisition and Analysis

Setting up a run in QuantaSoft Software

1. Upon completion of thermal cycling, transfer the sealed Droplet Plate to the QX200 Droplet Reader.
2. From the computer connected to the Droplet Reader, open QuantaSoft Software in the setup mode and Select New Run ⟶ Select Test Name ⟶ Select New Plate.

   NOTE: The instructions included in this section are applicable for both QX200 AutoDG Droplet Digital PCR Systems.
3. Double-click on a well in the plate layout to open the Well Editor dialog box. Select the wells appropriate for the run and choose the following (Figure 5):
   a. Experiment: ABS
   b. Supermix: One-Step RT-ddPCR Kit for Probes
   c. Target 1 Type: Ch1 Unknown
   d. Target 2 Type: Ch2 Unknown
4. Designate the sample names by well based on the plate layout.

Figure 5: QuantaSoft Setup: Well Editor Dialog Box

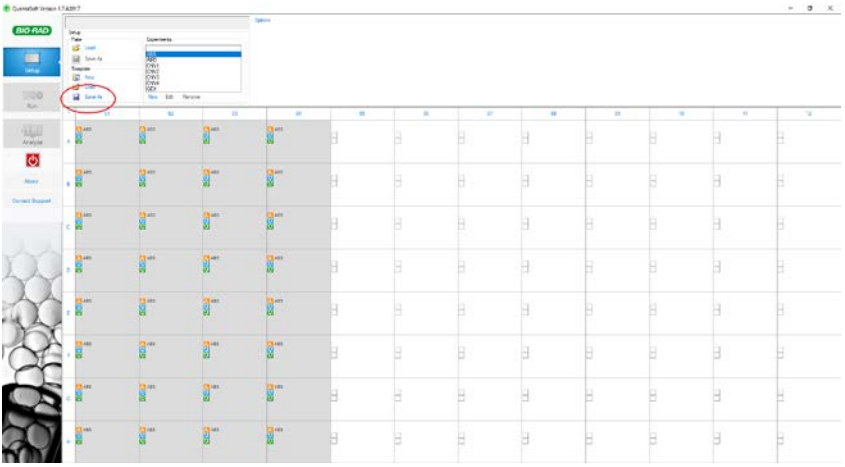


5. For each well on the plate, enter the Sample ID and select Apply.
6. Select the Sample Type for each of the wells.
    a. For wells that contain buffer, rather than a sample, select Buffer and touch Apply.
7. After finishing with well-name designations and the plate setup is complete, select OK.
8. Save a name for the plate by clicking Save As under the Template heading in Setup (Figure 6).
9. Prime the Droplet Reader by clicking the button on the upper right-hand side of the Setup mode window. It is recommended to prime the instrument before the first run each day.
10. Click the Run button from the left-hand panel to begin the droplet reading process.
11. On the right side of the screen, instrument status indicators should display green checkmarks for the system mechanical checks.

    WARNING: If any of the instrument status lights are not green, follow the instructions provided on the tool tip of the status.

12. During data acquisition the software will display information on preliminary quality of acquired data and expected time to completion.

Figure 6: QuantaSoft Setup: Saving a Completed Template

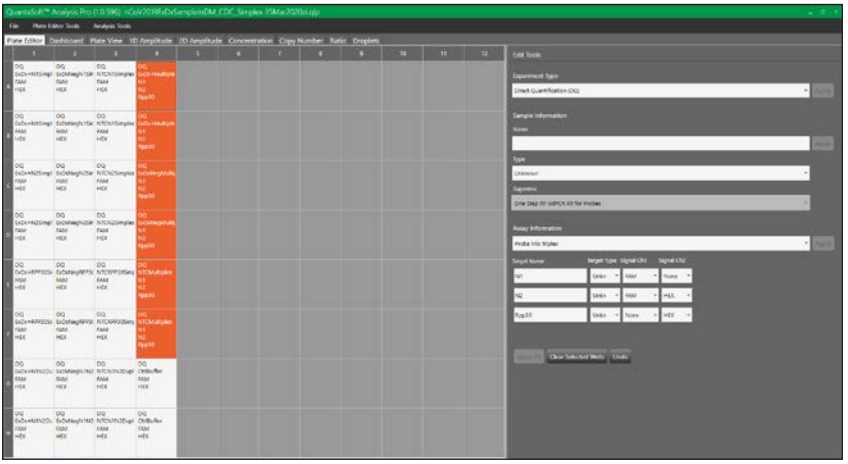


Data Analysis Using QuantaSoft Analysis Pro

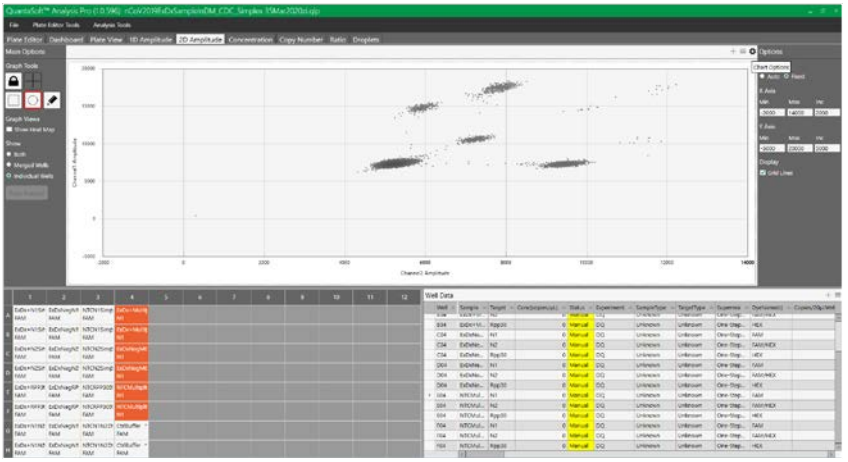
1. QuantaSoft Analysis Pro (AP) software is available to download and install at http://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system. Click on the Download tab. Please refer to the minimum system requirements before installation.
2. QuantaSoft Analysis Pro (AP) software opens and analyzes files generated by QuantaSoft Software 1.4 and later. This software is for analysis only; it cannot be used to run the QX200 Droplet Reader.
3. After Droplet Reading has completed, right-click the file for analysis and select Open with. Select QuantaSoftAnalysisPro. Alternatively, open QSAP by double-clicking the shortcut on the desktop or through the start menu then drag and drop the .qlp files to be analyzed into the analysis pro window.
4. In the Plate Editor tab, select all the wells to be analyzed. With Experiment Type as Direct Quantification (DQ); select Probe Mix Triplex under Assay Information and then click Apply. Enter Target information (Table 10) and click Apply.

Table 10: QuantaSoft Analysis Pro Target Information

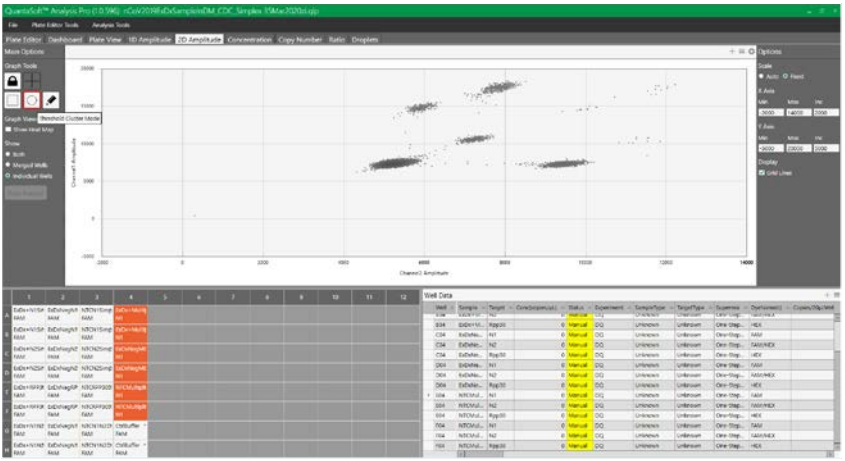
Target Name	Signal Ch1	Signal Ch2
N1	FAM	None
N2	FAM	HEX
RP	None	HEX



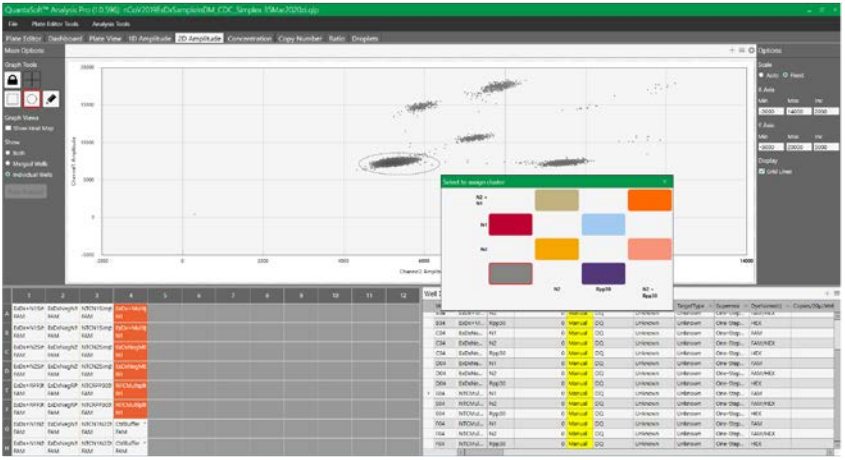
5. In the 2D Amplitude tab, select all the wells to be analyzed. Click the small gear icon to the right of the 2D plot and under Options and select Fixed. The 2D plot graph axes minimums and maximums can be adjusted to remove peripheral white spaces.



6. From the Graph Tools to the left of the 2D plot, select one of the Threshold Cluster Modes. Preferred mode can be selected based on user preferences; the Circular tool may be the most user-friendly. Keep Graph View at Individual Wells.

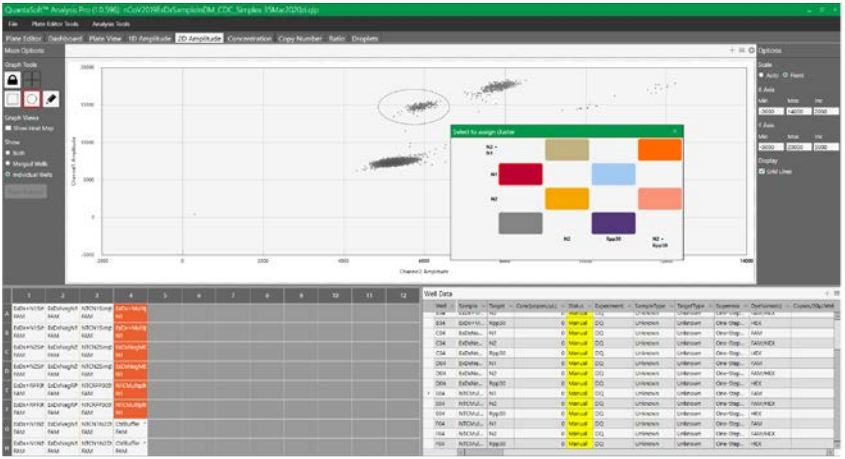


7. Using the mouse cursor, select the bottom left (triple negative) cluster. The Select to assign cluster window pops up. Choose the bottom left, grey assignment.

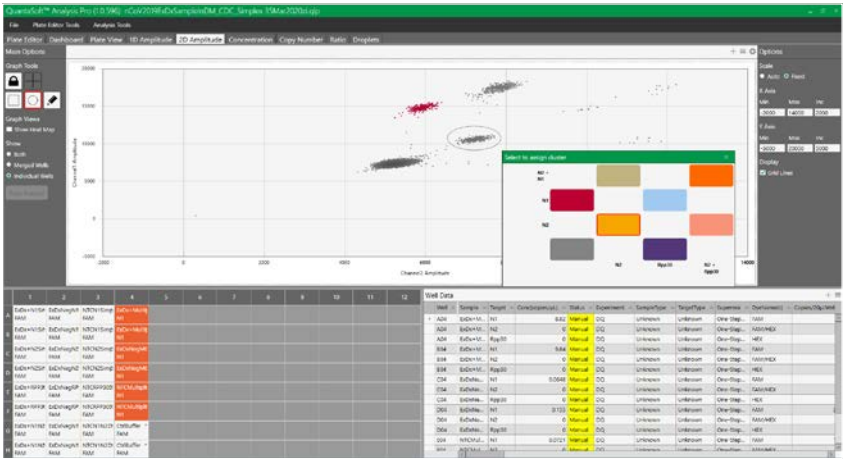


8. Repeat circling and assigning cluster definitions to all the clusters.

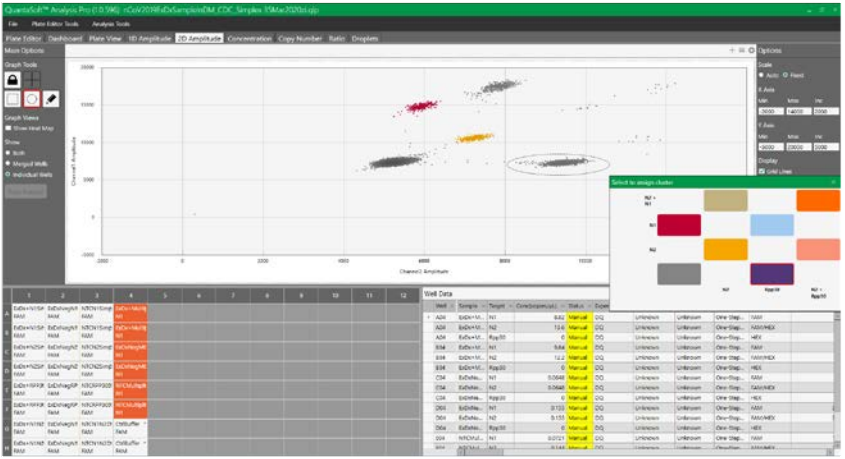
9. Cluster immediately above of the triple negative corresponds to N1 (single-positive, FAM channel).



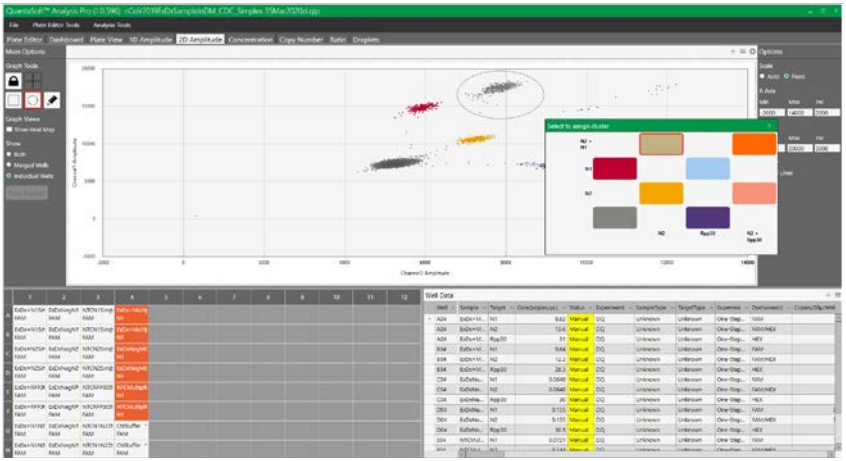
10. Cluster at a 45° angle from the triple negative corresponds to N2 (single-positive; FAM and HEX channels).



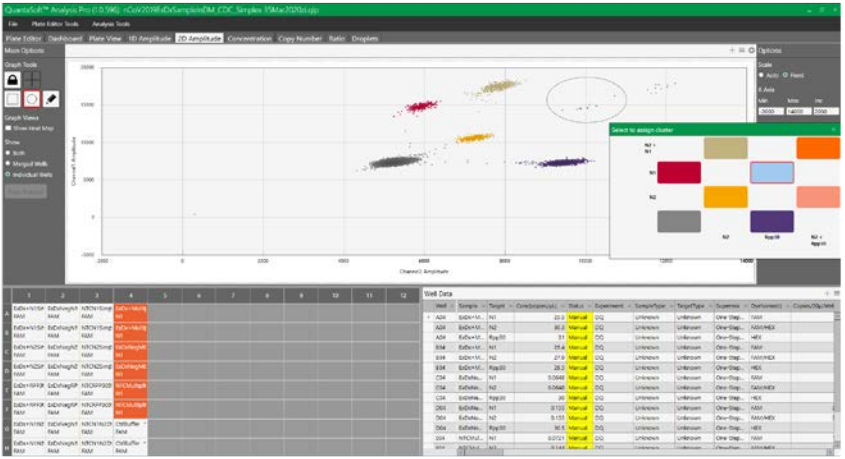
11. The cluster immediately to the right of the triple-negative cluster corresponds to RP (single-positive, HEX channel).



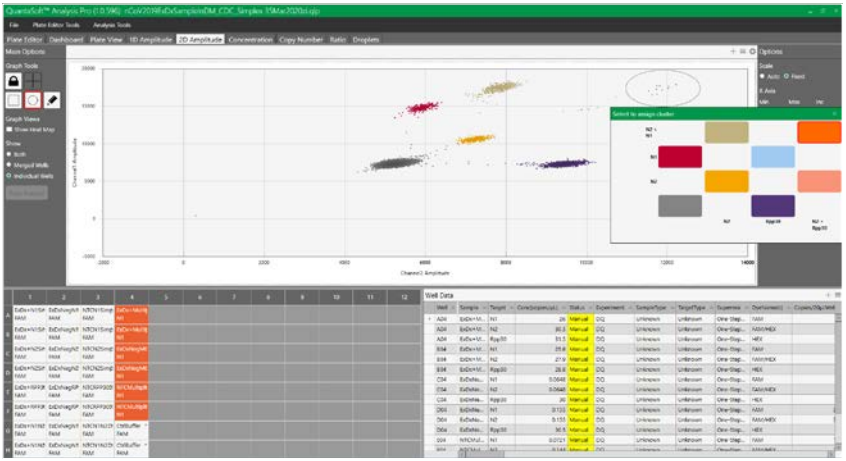
12. Above the N1 and N2 single-positive clusters is a double-positive cluster corresponding to droplets that are both N1 and N2 positive.



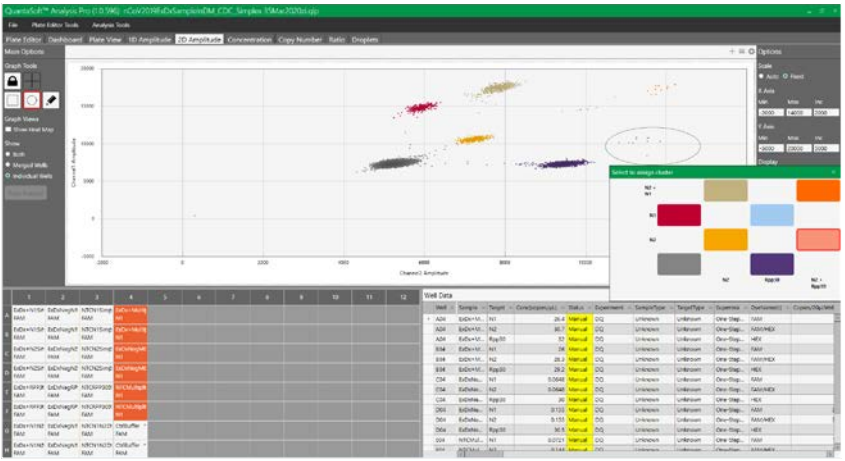
13. Above the RP single-positive cluster, there is a double-positive cluster corresponding to droplets that are both N1 and RP positive.



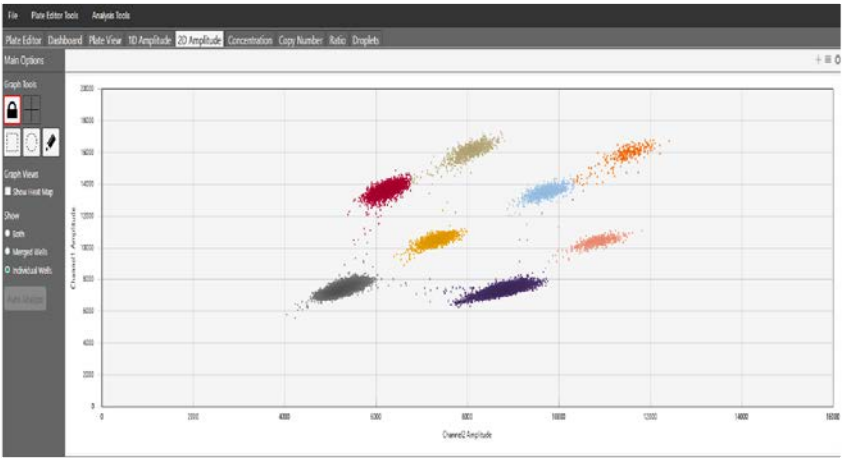
14. To the right of the N1 and N2 double-positive cluster there is an N1, N2 and RP triple-positive cluster corresponding to droplets that are positive for all three targets.



15. Next page, the triple-positive cluster there is an N2 and RP double-positive cluster corresponding to droplets that are both N2 and RP positive



16. Cluster assignments are applied upon selection. The final configuration will look like the image below.



NOTE: After applying cluster designation on an entire plate basis, it is essential to select each well and inspect corresponding clusters. Use the circular to Threshold Cluster Mode tool to reassign tight clusters on a per-well basis and check for inaccurate clustering or clusters that weren't entirely selected.

17. Quantifications are provided in the Well Data window on the lower right. Use the triple-bar icon on the upper right hand of the Well Data table to export data to Excel/csv.
18. The concentration is stated in copies/µL of the final 1X ddPCR reaction for each of the targets (N1, N2 and RP).
    a. Authorized laboratories will collect information on the performance of your product and report

REFERENCES

1. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer statistics, 2018. CA Cancer J Clin, 2018. 68(1): p. 7-30.

NOTES:

NOTES:

NOTES:

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site bio-rad.com **USA** 1 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 43 01 877 89019 **Belgium** 32 03 710 53 00 **Brazil** 55 11 3065 7550 **Canada** 1 905 364 3435
China 86 21 6169 8500 **Czech Republic** 36 01 459 6192 **Denmark** 45 04 452 10 00 **Finland** 35 08 980 422 00 **France** 33 01 479 593 00 **Germany** 49 089 3188 4393
Hong Kong 852 2789 3300 **Hungary** 36 01 459 6190 **India** 91 124 4029300 **Israel** 972 03 963 6050 **Italy** 39 02 49486800 **Japan** 81 3 6361 7000 **Korea** 82 2 3473 4460
Mexico 52 555 488 7670 **The Netherlands** 31 0 318 540 666 **New Zealand** 64 9 415 2280 **Norway** 47 0 233 641 30 **Poland** 36 01 459 6191 **Portugal** 351 21 4727717 **Russia** 7 495 721 14 04
Singapore 65 6415 3188 **South Africa** 36 01 459 6193 **Spain** 34 091 49 06 580 **Sweden** 46 08 555 127 00 **Switzerland** 41 0617 17 9555 **Taiwan** 886 2 2578 7189 **Thailand** 66 2 651 8311
United Arab Emirates 971 4 8187300 **United Kingdom** 44 01923 47 1301

Sig 0119